LARVAL TREMATODES FROM AUSTRALIAN FRESHWATER MOLLUSCS PART XII

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(Fig. 1-8)

[Read 13 November 1947]

The present paper deals with two new furcocercariae which have been rarely met with by us. One, *Cercaria ancyli*, belongs to the Strigeids, while the other, *C. lophosoma*, is a lophocercaria whose unknown adult probably is a blood fluke inhabiting one of the species of fish occurring in the Murray River.

We desire to acknowledge our indebtedness to Mr. G. G. Jaensch and his family for help during our visits to Tailem Bend; and to the Commonwealth Research Grant for financial assistance. Part XI of the series is being published in the Records of the South Australian Museum, 1947, 563-584.

Cercaria ancyli n. sp.

(Fig. 1-3)

A very small furcocercaria, *Cercaria ancyli*, has been found parasitising two different hosts, the gastropod *Amerianna pyramidata*, and the freshwater limpet, *Ancylus australicus*. It is the first occasion on which we have found larval trematodes in the latter mollusc. The cercaria was first observed as an infection of one out of six *Ancylus* collected in the River Murray swamps at Tailem Bend in April 1947. In May 1947 three out of 154 *Amerianna* from these swamps emitted the same kind of cercaria. It is possible that, owing to the superficial resemblance of this species to *C. angelae* Johnston and Simpson 1944, the former may have been collected on earlier occasions, but confused with the latter parasite.

The cercariae are very active and swim, tail first, almost constantly. They live for about 36 hours at room temperature, but keeping them in a refrigerator, at about 1° Centigrade, in a vessel of water, prolongs the life to two or three days, a useful expedient, as material was scarce.

Ten cercariae from each host, preserved in the usual manner, were measured with an ocular micrometer from a water mount. The measurements of the two lots of cercariae differ slightly, particularly in the proportions of tail-stem length to body length; but both body and tail-stem are highly contractile, and as the cercariae possess no noticeable anatomical differences, the differences in the measurements may be accounted for by their development in different hosts.

Measurements are given in micra and indicate the average, and (in brackets) the range. Cercariae from *Ancylus*:—body length, 114 (81-144); body breadth, 33 (27-41); tail-stem length, 112 (90-127); tail-stem breadth, 36 (27-41); furca length, 114 (99-124); furca breadth, 21 (18-25); anterior organ length, 34 (25-43); anterior organ breadth, 22 (18-25); ventral sucker length, 18·7 (18-21); ventral sucker breadth, 17 (14-19). Cercariae from *Amerianna*:—body length, 127 (93-153); body breadth, 37 (30-35); tail-stem length, 88 (72-100); tail-stem breadth, 35 (28-45); furca length, 116 (95-139); furca breadth, 21 (19-23); anterior organ length, 32 (27-37); anterior organ breadth, 25·5 (25-27); ventral sucker length, 18·7 (18-21); ventral sucker breadth, 19 (18-21).

The spines are restricted to the anterior organ and ventral sucker. In front of the mouth are three rows of forwardly directed spines, ten to twelve in all,

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with usually only one or two in the most anterior row (fig. 1) and four to five in the most posterior. A short spineless area succeeds the pre-oral spines, followed by a band of five or six irregular rows of spines around the front part of the anterior organ. The portion of the anterior organ anterior to the first row of spines may be completely withdrawn, or pushed forward. The ventral sucker bears three rows of rather irregularly arranged spines, approximately 50 in number, and these, too, can be withdrawn into the cavity of the sucker.

The digestive system consists of mouth, surrounded by the pear-shaped, highly contractile, anterior organ; a short pre-pharynx; well-developed pharynx; very short oesophagus; and a short knob-like caecum, all in the anterior third of the body. The caecum is at times partly lobulated in a manner suggestive of incipient division into two caeca (fig. 1).

There is a group of six rather small penetration gland cells posterior to the ventral sucker, arranged in two groups overlapping anteriorly. The ducts of these pass forward and open each side of the mouth. There is a group of probably four pairs of very small "head-glands" in the region of the anterior organ, which stain very deeply with neutral red used intra-vitam, and are hence conspicuous features in such a preparation. The genital primordium is a triangular mass of undifferentiated cells between the bladder and the two sets of penetration gland-cells. The nervous system was not observed. A large number of very small, highly refringent granules are scattered throughout the body. Whether these are part of the developing excretory system is uncertain.

The stem of the longifurcate tail contains six pairs of caudal bodies, the first rather smaller than the others. The usual stalked cells line the borders of the tail-stem. Both tail-stem and furcae are spineless. Transverse and longitudinal muscle fibres are present. A unique feature of this cercaria is the curious cuticular thickening halfway along the furca, opposite the opening of the excretory canal. This is knob-like in some, in others more spur-like, and is invariably present (fig. 2).

The bladder is trilobed, consisting of a central portion with an antero-lateral lobe on each side (fig. 1). Into this lateral lobe on either side opens the main collecting duct, which receives two secondary ducts at the level of the ventral sucker. The most anterior of these ducts drains the capillaries of two flame-cells, while the posterior, which is greatly coiled proximally, receives the capillaries of two further flame-cells in the body, and one in the tail at the level of the second pair of caudal bodies. Hence the excretory formula is $2[(2)+(2+(1))]=10$. In the region where the main ducts receive the secondaries a transverse commissure connects the two sides of the excretory system, passing across the body posterior to the ventral sucker. From the posterior part of the bladder a wide duct leads back, dividing to surround a small island of Cort, then continuing centrally along the tail-stem (fig. 3) and branching at the base of the furcae into two vessels which open halfway along the furcae, opposite the cuticular "spurs."

In one specimen a variation in the number of flame-cells on one side was observed. A third flame-cell in the hinder part of the body was connected with the posterior secondary duct on one side only, making the formula for that side $[(2)+(1+2+(1))]=6$ (fig. 1). This was apparently a precociously developed flame-cell of the metacercarial stage.

Sporocyst

The sporocysts (fig. 3) occur in the digestive gland in both hosts. They are slender tubular structures, usually tangled together in masses. Much of the liver

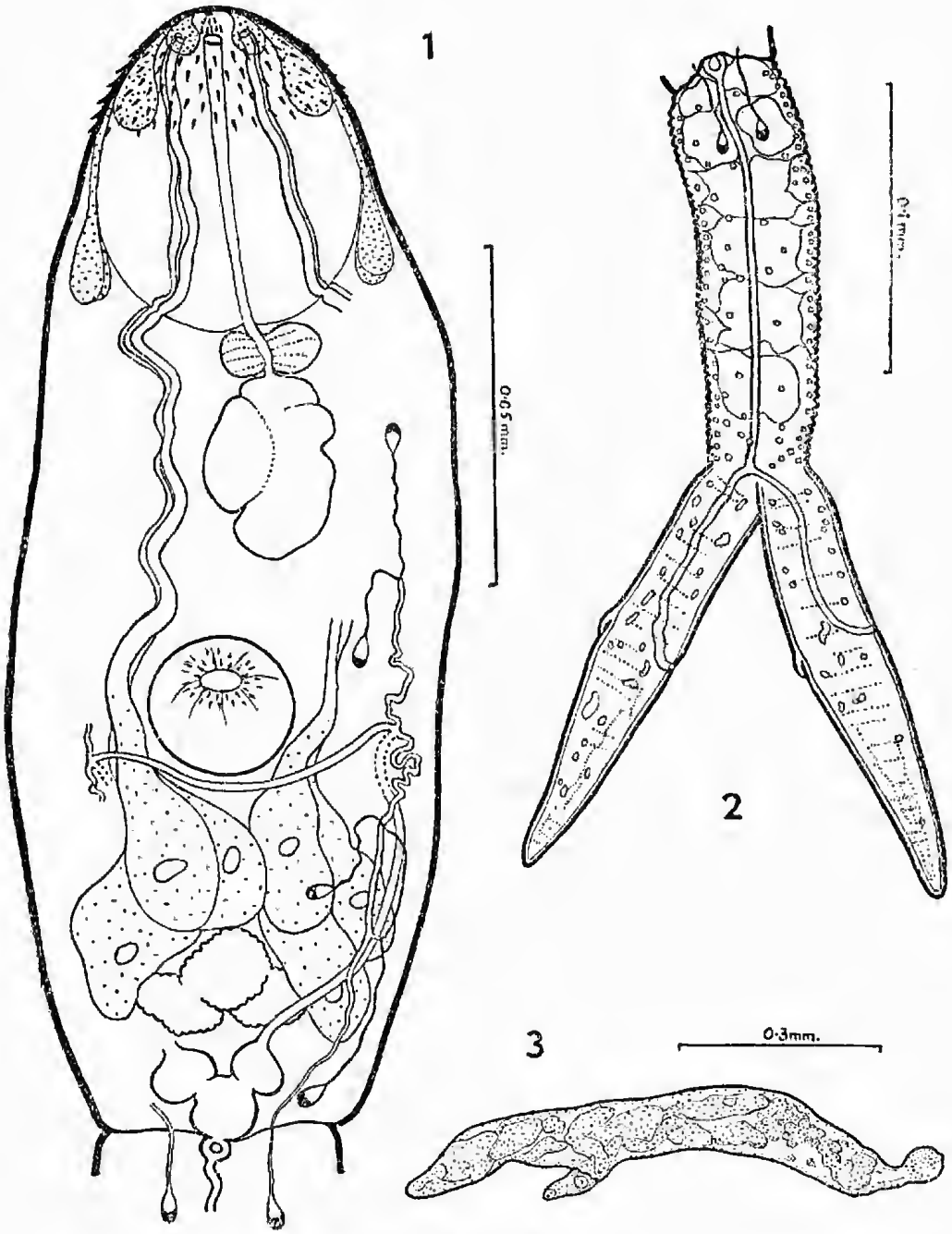

Fig. 1-3, *C. ancyli*

1, body, showing spines, digestive system, glands, excretory system, genital primordium; 2, tail; 3, sporocyst, showing cercaria emerging from tear in wall. Fig. 1 and 2 drawn from living specimens, outlines with camera lucida; fig. 3 from Canada balsam mount, also with camera lucida.

tissue of the host may be destroyed. The living sporocyst is capable of slow waving movements. Several sporocysts contained numbers of mature cercariae at the time when the first host (*Ancylus*) died, and some of the cercariae were observed pushing their way out through the walls of the sporocysts, but there is

apparently no birth-pore. Characteristically, a number of constrictions divides each sporocyst into several lobes, and the end may be marked by a small knob. Length is very variable—a fairly long one measured 3 mm. There are no very marked differences between sporocysts from the two hosts, but those from *Amerianna* tend to be slightly stouter and more coiled than those from the smaller host, *Ancylus*.

Experimental Infections

The second intermediate host of *C. ancyli* has not been ascertained. Attempts were made to infect experimentally with the cercaria the gastropods *Lymnaea lessoni*, *Amerianna pyramidata* and *Planorbis isingi;* the fish *Gambusia affinis*, and mosquito larvae; but results were in every case negative.

Relationships

As far as has been possible to ascertain, *C. ancyli* is the first cercaria from a fresh-water limpet to be described fully. Fielder, in 1896, mentioned the presence of cercariae and of pigmented distome cysts in *Ancylus tasmaniensis;* while Cherry, in 1895, reported having seen cercariae from the same species, and in 1917 from *A. australicus*. As these cercariae were not described, it is impossible to know whether any of them could have been *C. ancyli*. The occurrence of this cercaria in *Amerianna* as well, indicates that its nearest relatives need not necessarily be parasites of fresh-water limpets.

One local cercaria, *C. angelae*, Johnston and Simpson 1944, also from *Amerianna*, resembles *C. ancyli* closely enough to make identification troublesome in routine examinations. The two species are distinguished, however, by a number of features, namely, size—*C. angelae* is considerably larger; number of gland-cells—eight in *C. angelae*, six in *C. ancyli;* number of flame-cells—ten in *C. ancyli*, sixteen in *C. angelae;* and absence of "preacetabular bodies" in *C. ancyli*. They are also distinguished by the form of the alimentary canal, the spination, and by the fact that the tail excretory tubules in *C. ancyli* open half-way along the furcae, but in *C. angelae* at the tips.

Several well-defined groups of cercariae can be eliminated immediately from close relationship with *C. ancyli*, although possessing some features in common with the latter. The *Elvae* group of furcocercariae (Miller 1923) all possess a single pair of caudal flame-cells high in the tail-stem, apparently a group characteristic rather than a species characteristic, and common to several groups of furcocercariae; but they differ from *C. ancyli* in various fundamental features, such as the possession of a brevifurcate tail. Probably much more closely related, though still distinct, is the *Apatemon* group, again with one pair of flame-cells in the tail-stem, and also resembling our larva in size, general proportions and spination, but differing in having four pairs of gland-cells, seven pairs of flame-cells, and well-developed caeca. *C. multicellulata* Miller (1923) and its allies possess six gland cells posteriorly placed, but are quite distinct from *C. ancyli* as a group, as they have two pairs of flame-cells in the tail-stem, a larger number of flame-cells in the body, and differ greatly in size, spine equipment and other features.

The cercaria mostly closely resembling ours is *C. dohema* Cort and Brackett (1937), a parasite of *Lymnaea* and *Stagnicola*. Of the relationships of this cercaria, Cort and Brackett said (p. 278) that they could find in the literature no other strigeid larva resembling theirs. *C. ancyli* has exactly the same excretory arrangement, similar type of gut and glandular equipment, similar pre-oral spines, six caudal bodies in the tailstem and somewhat similar body proportions. In actual size, however, *C. dohema* is rather larger (body 156 μ long, tail-stem 179 μ

long, furca 196 μ long); it also differs in having spines back to the level of the pharynx, unpigmented "eyespots," a pair of caudal bodies in the furcae, no head-glands and no furcal spur, and its sporocyst possesses a birth-pore. *C. dohema* is said to penetrate into a minnow (*Fundulus* sp.), and metacercariae (undescribed) recovered from the liver were considered to belong to that species, but investigations of the life-cycle were not completed.

C. riponi Brackett (1939) resembles *C. ancyli* somewhat less closely. The glandular equipment is very similar, and the excretory system differs only in the presence of an additional pair of flame-cells in the posterior body; but it has no pre-oral spines, more extensive body spination, well-developed gut, eyespots and no head-glands. *C. sincera* Olivier (1941) has an excretory formula identical with that of *C. ancyli*, and is very similar in size, but has only two pairs of glands, a heavily spinose body, and long caeca. *C. granula* Miller (1927) and *C. hirsuta* Miller (1927) are two more species with exactly the same excretory arrangements as *C. ancyli;* in *C. granula* there are also occasional variations in the number of flame-cells in the posterior part of the body, as in our larva. Both of Miller's cercariae have, in addition, very short caeca, and six caudal bodies, but differ from our larva in size, and markedly in their penetration gland equipment, although in both this is mainly posterior to the ventral sucker. Both have setae on the tail-stem.

Two other cercariae with six post-acetabular gland-cells, *C. higginsi* Olivier (1942) and *C. wallooni* Olivier (1941), are quite distinct from *C. ancyli;* both are considerably larger larvae than ours, and both have sixteen flame-cells, two of which are in the tail-stem.

Cercaria lophosoma n. sp.

(Fig. 4-8)

In the course of examination of 2,920 specimens of the gastropod, *Notopala hanleyi*, for trematode infestation, a minute new lophocercaria, *Cercaria lophosoma*, has been recovered from two snails. These two infected specimens were collected in May 1945 and March 1946 respectively at Swan Reach on the River Murray; ten other collections of this mollusc made at the same place between April 1942 and March 1947, and two collections made at Renmark and Morgan respectively, yielded no further specimens infected with the same cercaria. It is possible that the cercariae at times escape notice because of their small size.

C. lophosoma is emitted mainly in the middle of the day. A few appear by 10.30 a.m., but large numbers are not emitted until between 12 noon and 2.30 p.m. When sufficient numbers are present, they tend to form a swarm in the tube. They are planktonic organisms and swim very little, but will respond to vibration of their tube with a few jerky bending movements of the tail. They float in various positions (fig. 6). The length of life is about 48 hours.

Cercariae, fixed by adding an equal quantity of boiling 10% formalin to the water in which they were swimming, were measured in a water mount with an ocular micrometer. The measurement of the breadth of the furcae excludes the fins, which in preserved material are often bent or shrunk. The averages of ten measurements are given in micra, with the ranges in brackets: body length, 95 (82-108); body breadth at widest part, 27 (21-32); tail-stem length, 196 (180-213); tail-stem breadth, 19 (16-21); furca length, 63 (54-73); furca breadth, 7 (5-9); anterior organ length, 18 (16-19); anterior organ breadth, 16 (14-19).

A difficulty encountered in studying this cercaria was the impossibility of making a ventral mount during life, partly because of the lateral compression of the body, and partly because of the stiff cuticular crest. Hence the measurement given as "body breadth" is more exactly "body depth."

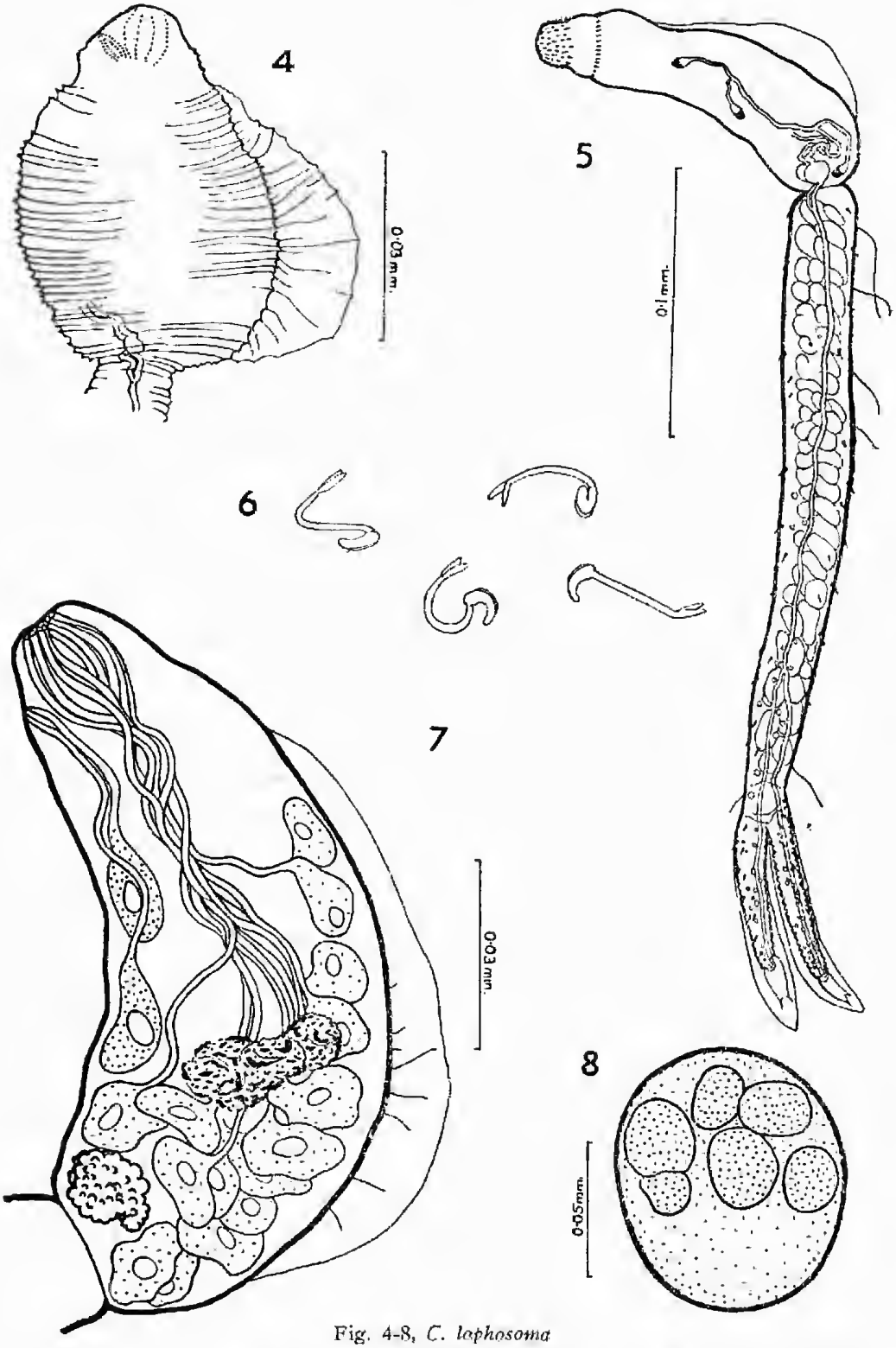

Fig. 4-8, *C. lophosoma*

4, body greatly contracted; 5, general features of body and tail, and excretory system; 6, various positions assumed when floating; 7, body in extended position, showing glands, central granular mass, and genital primordium; 8, sporocyst. Fig. 4, 5, and 7 drawn from living specimens, outlines with camera lucida; fig. 6 drawn freehand from living specimens in a drop of water without coverslip; fig. 8 drawn with camera lucida from Canada balsam mount.

The body of the cercaria is, like that of *C. helvetica xvi* (Dubois 1927, p. 27) highly contractile. There are five to six rows of fine straight spines round the front of the anterior organ. There are no further spines on the body, although when greatly contracted it has a spiny appearance, due to the intense wrinkling of the rather stiff cuticle (fig. 4). Small spines are scattered along the borders of the tail-stem, and more thickly on the fleshy part of the furcae.

The anterior organ is highly contractile and, as in Sewell's Indian Lophocercariae (1922, p. 46), definitely snout-like. There are no "hollow, conical spines" on the tip of this snout, as described for several Lophocercariae, but at times drops of secretion from the gland-ducts which open on the anterior surface of this organ, and which are highly refractive, may be seen. There is no ventral sucker. There are no eyes, though they have been described for some Lophocercariae.

The glands (fig. 7) are numerous, mainly in the middle and posterior regions of the body. Neutral red and Nile blue sulphate were used as intra-vitam stains, Delafield's haematoxylin and acetic acid alum carmine for permanent preparations. Two unicellular glands in tandem are situated ventrally, their ducts passing forward to open on the ventral surface of the anterior organ, separate from the other ducts. These two glands stain deeply with both neutral red and Nile blue sulphate, while a large group of glands dorsal to, and extending posterior to them, stain more lightly with neutral red, but just as deeply with Nile blue sulphate. These glands could not be accurately counted, but number more than twelve. Their ducts pass forward together centrally, to open on the apex of the snout. In the middle part of the body is a group of deeply-staining granules surrounded by a granular mass. It could not be determined whether these were the rudiments of the ventral sucker, or part of the genital primordium. A triangular-shaped mass of cells, staining deeply with acid alum carmine and with Delafield, situated just in front of the bladder, is certainly part of the genital primordium.

No trace of a digestive system is present; not even a mouth-opening was observed. The nervous system could not be distinguished.

The dorsal crest is a conspicuous feature of this cercaria. It is fine, transparent and slightly yellow, and is apparently formed of an extension of the cuticle. Its shape varies with the extension and contraction of the body (fig. 4 and 7).

The long slender tail is, together with the furcae, two to three times as long as the body (fig. 5). It is provided with a few very fine, hair-like structures dorsally, as well as the minute spines mentioned above. There are numerous small rounded caudal bodies grouped round the central axis of the tail; these disintegrate readily under pressure. A few scattered nuclei in the tail-stem stain deeply with neutral red in life. The tail-musculature is well developed. The principal fibres run obliquely in two directions, and hence in two sets, one dorsal and one ventral; when viewed from the side they appear to be arranged in herring-bone fashion, the apices of the one set directed forward, those of the other backward. Longitudinal fibres also are present.

Each short slender furca is provided with a fine cuticular flange or fin, extending round the whole furca. Longitudinal muscle fibres are present. At the end of the furca the flange forms a pocket-like or flask-like fold, open posteriorly, as is characteristic for this type of cercaria. At the base of this pocket, on the tip of the furca, opens an excretory pore.

As far as could be determined before the host died, the excretory system is of the usual pattern, *i.e.*, $2\ (2 + 1) = 6$. The two sides of the excretory system,

if it be bilateral, were, however, never seen simultaneously in one specimen. The bladder (fig. 5) is bilobed, and from each side a duct passes forward. From this point, presumably owing to the fact that a ventral view was never obtained, only one set of tubules could be seen. The main duct branches into two, one passing forward to receive the capillaries of two flame-cells, the other passing back to receive a single flame-cell at the level of the bladder. There are no flame-cells in the tail. There is a long island of Cort, from which a single duct passes back through the clusters of caudal bodies, to divide into two tubules about three-quarters of the way back along the tail-stem (fig. 5). One tubule passes into each furca, opening at the tip into the flask-like extension of the flange.

Experimental Infections

Unsuccessful attempts have been made to infect the fish, *Gambusia affinis* and *Carassius auratus*, and a tortoise, *Emydura macquarii*, with the cercaria.

Sporocyst

The sporocysts are very small, round, or oval bodies (fig. 8), and at the time of examination, after the death of the host, contained only germ-balls, which stained more deeply with acid alum carmine than did the rest of the sporocyst. Large masses of these parthenitae were packed together in the liver. They vary somewhat in size, and also in shape, according to the pressure of the surrounding tissue. The average length of ten, measured with an ocular micrometer from a canada balsam mount of a stained fragment of liver, was 101 μ, varying between 84 μ and 120 μ.

Relationships

The "Lophocerca" group of furcocercariae, so named by Lühe in 1909 to include *C. cristata* La Valette and *C. microcristata* Ercolani, was defined fully by Sewell in his "Cercariae Indicae" (1922); the essential features of cercariae of his "Lophocerca" group being the very small size, the relatively long, brevifurcate tail with furcae provided with a fin-fold; body with a crest; eyes (in many); gland cells in mid-body; anterior organ snout-like rather than sucker-like; no mouth, alimentary canal or ventral sucker; excretory formula, $2\,(2+1)=6$; development in small oval or rounded sporocysts. Sewell himself added four cercariae to this group (*Cercariae indicae IX, XIII, XXXIX*, and *LV*), and since then a number of other Lophocercariae have been described.

Odhner (1911), Scheuring (1922), and Ejsmont (1925), have shown that certain forms belonging to this group develop in the blood of Cyprinid fish into species of the genus *Sanguinicola*. Wall (1939; 1940), on the other hand, described a cercaria possessing the characteristic dorsal crest, which developed into *Spirorchis parvus* Stunkard, a blood-fluke of American freshwater tortoises. However, the life-history of another species of *Spirorchis*, *S. elephantis* Cort, was described by Wall in 1941, and its cercaria possesses no dorsal crest. Thus, if the possession of a dorsal crest be the only characteristic necessary for inclusion of a cercaria in the Lophocerca group, it would at once become a highly artificial group, closely related forms being separated, and unrelated forms being grouped together. Under Sewell's more limiting definition the presence of a dorsal crest is only one of several distinguishing features, and the cercaria of *Spirorchis parvus* is excluded from the group by the possession of an alimentary canal (apharyngeal) and a ventral sucker, and by its excretory formula of $2\,[\,(1+1+1)+(\,1+1+(1)\,)\,]=12$. Furthermore, Wall's description of the crest of the cercaria of *S. parvus* indicates that its structure is somewhat different from that of the true Lophocercariae.

C. lophosoma is a Lophocercaria belonging to Sewell's group, but distinct from all other members. In size it is closest to *C. helvetica XVI* Dubois (1929), which, like our cercaria, has numerous gland cells and no eyes. Complete comparison is not possible because Dubois' description is very brief; the host of Dubois' larva is a very different gastropod, however, *viz.*, *Lymnaea*. *C. lophosoma* differs from Sewell's *Cercaria indica IX* (from *Indoplanorbis* and *Gyraulus*), *XXXIX* (from *Amnicola*), and *LV* (from *Amnicola*), in having no hollow spines on the tip of the snout, no eyes (pigmented or otherwise), a greater number of glands cells distributed differently, and finally, in size—being slightly larger than *C. indica IX*, and somewhat smaller than either *C. indica XXXIX* or *C. indica LV*. *C. indica XIII* (from *Amnicola* and *Melanoides*) has no apical spines, but is larger than *C. lophosoma*, possesses non-pigmented eyespots, fewer gland cells, no spines on the furcae, and a papilla-like structure ventrally, thought to be a rudimentary genital papilla. The body of Scheuring's *C. Sanguinicolae inermis* (from *Lymnaea*) is slightly larger, according to Ejsmont's figures, and the tail is larger in proportion to the body than in our cercaria. The measurements Ejsmont gives for his *C. Sanguinicolae* spp. from *Bithynia* and from *Lymnaea* are both somewhat larger than those of *C. lophosoma;* he shows two excretory canals in the tail-stem, and though the large number of gland cells is suggestive of our cercaria, Ejsmont figures also a short, blindly-ending gut, in a somewhat similar position to the two special gland cells of *C. lophosoma*.

C. cristata La Valette (1852), from *Lymnaea*, which Sewell considered (p. 53) might be the same organism as his *C. indica XIII*, is a larger cercaria than ours, according to Ejsmont's figures; the cercaria which Wesenberg-Lund (1934) describes as *C. cristata* has the same body measurement as *C. lophosoma*, but the tail-stem is longer and the furcae very much longer; there are special apical spines, two excretory canals in the tail-stem, and the sporocysts are long and provided with a sucking disc. *C. microcristata* (from *Bithynia*) is, according to figures given by Ejsmont, smaller than *C. lophosoma* and all other known Lophocercariae.

Martin (1944) redescribed a marine Lophocercaria, previously described by Linton (1915), and named *C. loossi* by Stunkard (1929). This larva is remarkable for having as its host an annelid worm, *Hydroides*. In all other respects it is closely allied to the Lophocerca group, and the sporocysts are apparently of the characteristic type. Both Linton and Martin consider it closely related to cercariae of the genus *Sanguinicola*. It is, however, quite distinct from *C. lophosoma* in being larger, but with furcae shorter, and in having twelve to thirteen rows of spines around the snout, and fewer glands, arranged differently.

C. lophosoma is distinct from *C. sewelli* Faust (1926), from *Burnupia*, which is larger, has only two pairs of glands, a pair of partly-pigmented eyes, and as far as can be seen from the figure, no fin-folds on the furcae. Three other dorsally-crested cercariae, *C. whitentoni* Croft 1933, *C. brevifurca* McCoy (1926) from *Planorbis*, and *C. bombayensis* No. 8 Soparkar (1921) from *Planorbis* and *Lymnaea*, show somewhat dubious affinities with true Lophocercariae, because all possess simple, apharyngeal alimentary canals, and fin-less furcae, and they develop in rediae. *C. bombayensis* No. 8 and *C. whitentoni* possess also a rudimentary ventral sucker, and the excretory systems are more complex than that of the true Lophocerca type. It is possible that these three cercariae may be more closely related to *Spirorchis* or perhaps to *Clinostomum*, since the cercaria of *Clinostomum marginatum* has a dorsal crest, finless furcae, alimentary canal, ventral sucker rudiment, and five pairs of flame cells, and develops in rediae Krull 1934).

Summary

Cercaria ancyli n. sp., a parasite of *Ancylus australicus* and *Amerianna pyramidata*, is a longifurcate pharyngeal strigeid distome cercaria with six penetration glands, head glands, ten flame cells, an excretory commissure behind the ventral sucker, very short gut, and a furcal spur. Metacercaria and life cycle are unknown.

Cercaria lophosoma n. sp., a parasite of *Notopala hanleyi*, is a dorsally-crested brevifurcate, non-ocellate cercaria with numerous unicellular glands, but without a ventral sucker or alimentary canal. It belongs to Sewell's group, Lophocercaria, and is closely related to the cercaria of *Sanguinicola*.

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